SECCIÓN VI.
CIENCIAS QUIMICAS

DOI 10.36074/24.04.2020.v3.02

IMMUNOCHEMICAL METHODS FOR STERIGMATOCYSTIN DETECTION

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Molds are ubiquitous in nature. Under certain environmental conditions moulds produce secondary metabolites. Those that are toxic to animals and humans are called mycotoxins. Mycotoxins are feared food contaminants, which have a negative impact on public health, food security and safety and the economy in many countries, particularly in developing ones. Some mycotoxins exhibit genotoxic, mutagenic, immunosuppressive, carcinogenic and teratogenic effects. To prevent health hazard in humans and animals regular monitoring and control of feed and food occur in almost all countries. There are two different ways in which moulds could affect the humans or animals health. Inhaling of fungal spores could cause diverse allergic reactions or systemic mycosis. The second route of exposure occurs via the digestive tract, due to consumption of food spoiled by mycotoxins. Depending on the amount and duration of exposure mycotoxin can cause acute or chronic toxic effects.

The major mycotoxin groups, which are monitored, are aflatoxins, ochratoxins, trichothecenes, patulin, fumonisins and zearalenon. Due to their high toxicity and carcinogenetic effect, aflatoxins is the most important one among these groups. Their discovery and further investigation appears rather late, in 1960. After this finding, a huge amount of time and effort was invested into toxicological studies, the development of methods for routine control in food and feed.

The mycotoxin sterigmatocystin (STC) is a precursor in the biosynthesis of aflatoxin B_1 (AFB_1). The mechanism of molecular action of STC is very similar to AFB_1. However STC seems to be less toxic than AFB_1.

Up to now there is no harmonised regulation for STC within the EU. Only the Czech Republic and Slovakia have set maximum allowed levels for STC.

Routine analysis of aflatoxins is preferably done by HPLC-FLD in all sectors dealing with food and feed. Therefore the development of a method for the simultaneous and sensitive determination of STC and AFBG would be very beneficial [1].

A lot of different methods for STC detection have been performed during last decades. The methods can be divided into two groups due to their mechanism: chromatographic [2] and immunochemical.

General working principle of immunochemical methods The Antibody-Antigen-interaction (Immunochemical principle) is used in the analytic of mycotoxins as a

quantitative method (ELISA) and also as a clean-up step (IAC) for extraction of desired molecule with further quantification by other methods.

The working mechanism of these methods is an immunological reaction between antigen and antibody to an immune complex. This immune complex can be further detected using enzyme reaction, if quantification is desired or broken and released for further quantification using other technics. The production of specific antibodies for the desired substance is the first step. Antibodies are proteins, consisting of four polypeptides and having property to recognise and tie the antigen. In the figure 1 an antibody consisting of the two heavy and the two light chains of polypeptides is illustrated schematically.

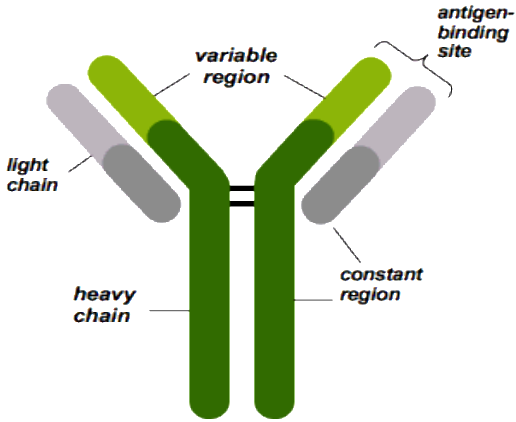

Fig. 1. **Scheme of antibody**

The variable region, due to its composition, gives the antibody this property to select, recognise and tie a desired substance (Antigen). To succeed in immune complex building, the antigen has to fulfil the following criteria: organic, high molecular weight (M_r > 10 000) substance, which would be recognised as foreign and can lead to antibody building against itself. Only high molecular weight antigens can have immunogenic properties. STC is a low molecular weight molecule. Therefore, for a successful production of antibodies against STC an attachment to a carrier-protein is required [3]. These technics about attaching the STC molecules to carrier-proteins are described in the literature [4, 5]. The way of attaching the carrier-protein can play a crucial role on the antibody specificity.

Antibody production includes the immunisation of a test animal with an antigen and further production of polyclonal or monoclonal antibodies. For polyclonal antibody production, antiserum is directly taken from the animal. This antiserum contains a mixture of antibodies from different spleen cells. For the monoclonal antibody production the spleen cells are isolated and crossbreed with myeloma cells. The desired hybridomas are selected and cloned. The achieved hybrid cells are checked in order to select one cell, producing the desired antibody. In the last years the production of monoclonal antibodies became more popular, due to its specificity and homogenous origin, although the production is more expensive.

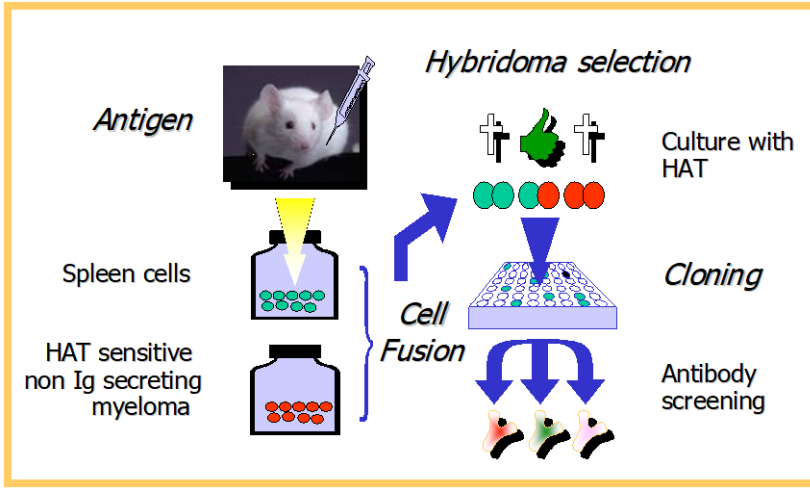

Fig. 2. **Monoclonal antibody production**

Immunological quantitative methods developed for STC (ELISA) A quantitative competitive Enzyme-Linked Immunosorbent Assays (ELISA) has been developed by Li et al. for the determination of STC-hemiacetal. The produced antisera were highly sensitive (LOD= 0.25 ng/ml) against the STC-hemicacetal, showed little cross-reactivity against dehydro-STC (16 times less sensitive than STC-hemiacetal) and practically no cross-reactivity with AFB_1, AFB_2, AFG_1, AFG_2 and AFM_1. To enable quantification, STC had to be converted to the hemiacetal form [4]. The production of antisera to STC-hemiacetal and a novel conjugation procedure for immunogenic synthesis is described by Morgan et. al. In this investigation the developed cELISA was validated for barley matrix [5]. Creation of a competitive ELISA based on a high-affinity monoclonal antibody, which requires no derivitization, was reported by Li et al in 1996 (LOD<0.5 ng/ml). The drawback of this method was a high cross-reactivity with AFB_1-hemiacetal (6.25%) and reduced cross-reactivity with STC-hemiacetal (12%) [6].

Advantages of immunochemical methods are the absence of a time consuming clean-up treatment of the sample and that there is no need to use expensive equipment for separation and detection. At the same time the development of an immunological assay is complex and time-consuming. The complex food matrixes require comprehensive validation and standardisation due to the possible occurrence of unwanted substances and their potential association with antibodies [7]. Since false positive results can occur, a confirmation by chromatographic methods is often required.

Immunoextraction The principle of the Immunoaffinity columns (IAC) is relatively simple. An antibody (polyclonal or monoclonal) raised against the analyte is immobilised on a gel, and generally about 0.2–0.5 ml of gel is packed into a small plastic column. The column is initially conditioned with phosphate buffered saline (PBS). Than a mainly aqueous sample extract is applied slowly to the column at around 1–2 ml/min. The sample can be applied under gravity or under positive pressure from a syringe or can be sucked through the column under vacuum [8].

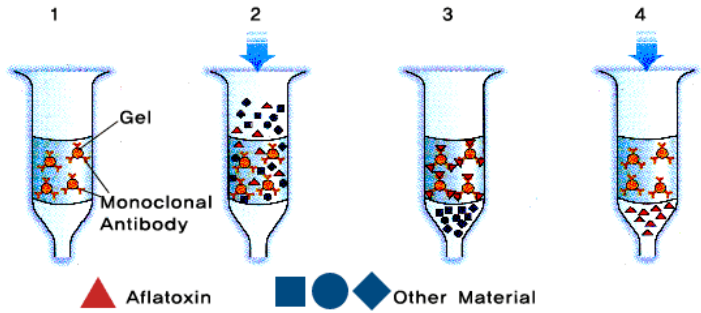

Figure 3. Working principle of IAC

Very important properties of antibodies are selectivity and cross-reactivity. In the immunological reaction between antibody and antigen differently strong binding forces are involved. These can result into binding reaction between antibody and structurally similar molecules. This effect is called cross-reactivity. This effect is not always considered as negative. In mycotoxin analyses the molecules of interest sometimes have a similar structure (for example AFB_1, AFB_2, AFG_1, AFG_2). In this case the cross-reactivity is desired and allows simultaneous determination of them. IAC's for mycotoxin extraction are commercially available by many producers.

Conclusion. The article mentions a number of experiments in which the mutagenicity and carcinogenic potential of a given chemical substance was evaluated. According to their results, STC was assigned to the group of the most powerful mutagens [1].

This indicates the need to monitor this mycotoxin in food and feed. Especially the occurrence of STC at low levels remains questionable, due to the high limit of detection of most methods used for its determination. Therefore, it is important to develop a sensitive method for determining STC along with other mycotoxins.

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DOI 10.36074/24.04.2020.v3.03

АДСОРБЦИЯ ДИКОМПОНЕНТНЫХ ИНГИБИРУЮЩИХ СМЕСЕЙ

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Учитывая структуру ряда исследованных полифункциональных пиридиновых солей, которые содержат пиридиновый и ацильный фрагменты, соединенные подвижной метиленовой группой [1], различные по строению и химическим свойствам заместители, полученные экспериментальные данные, на примере соединений, имеющих в пиридиновом кольце аминогруппу, изучено адсорбцию, созданных на их основе дикомпонентных ингибирующих смесей (ДИС).

При адсорбции исследованных ДИС на железе и сталях, несущих в растворе кислот отрицательный заряд поверхности, при 20°С часть соединений ориентируется по пиридиновому фрагменту, принимая планарное расположение, что обусловлено π-электронным взаимодействием пиридинового кольца солей с металлом [1-5]. Ацильная часть соединений, согласно пространственной конфигурации молекул, располагается при этом в объеме раствора с ориентацией отрицательно заряженного кислорода карбонильной группы [1] в сторону водной фазы.

Ориентационные расположения ДИС подтверждаются корреляционными зависимостями, свидетельствующими об увеличении ингибирующего действия соединений, при введении в пиридиновый фрагмент электронодонорных заместителей [4]. Такому расположению адсорбированных частиц ДИС на отрицательно заряженной поверхности способствует также наличие в пиридиновом кольце, способной к протонизации [2], аминогруппы, которая вносит дополнительный положительный заряд в электролитическую обкладку двойного электрического слоя.